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APPEARANCE OF INGESTED $H_2{}^{18}O$ IN PLASMA AND SWEAT

DURING EXERCISE-HEAT EXPOSURE

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APPEARANCE OF INGESTED $H_2^{18}O$ IN PLASMA AND SWEAT

DURING EXERCISE-HEAT EXPOSURE

(1 table, 2 figures)

Abstract

In an effort to study human water transport and eccrine sweat gland function, this investigation measured the rate of appearance of $H_2^{18}O$ in plasma and sweat. Four healthy males were exposed for 6 h to a hot, wet environment (37.1°C db, 31.3°C wb) and to intermittent cycle ergometer protocols. Baseline plasma (antecubital vein) and scraped sweat samples were collected (-30 to 0 min) prior to administration of 100 g of labelled water (84.7 % ^{16}O, 15.3 % ^{18}O) via nasogastric tube at 0 min. Samples were analyzed using isotopic ratio mass spectrometry. The isotopic enrichment of baseline sweat samples was slightly greater than that of plasma samples. Peak enrichment ($^{18}O/^{16}O$ ratio) in plasma (range: 2.4908 - 2.7206 x 10^{-3}) occurred at 21 - 28 min postdose and at 21 - 45 min postdose in sweat (range: 2.3089 - 2.6666 x 10^{-3}). The $^{18}O/^{16}O$ ratio in plasma and sweat declined rapidly, then declined slowly for the remaining heat exposure. The appearance of $H_2^{18}O$ in sweat reflected that of plasma; neither curve was significantly altered by exercise intensity, duration, or frequency. To our knowledge, this is the first stable isotope data to verify that ingested fluid is rapidly assimilated and becomes available for evaporative cooling during work in the heat.

KEY WORDS: stable isotope, blood plasma, sweat, $H_2^{18}O$, fluid transport, sweat gland

Introduction

Eccrine sweat glands have been studied for a variety of reasons (6,9,12,16), the most important of which is to measure the effects of sweat secretion on whole body fluid/electrolyte balance and thermal balance. Early studies focused on the effects of water intake on sweat rate; Lee and Mulder (8), for example, were the first to report that bursts of sweating could be observed approximately 3 minutes after water consumption. Similar bursts of sweating have been described by Kuno (6), Senay and Christensen (15), Banerjee and Bullard (3), and Lechner and Hertig (7). However, evidence is still lacking to determine whether these bursts represent a reflex response to fluid intake, or are a manifestation of altered pressure/volume relationships or osmolarity within the circulatory system. Researchers routinely have assumed that a sweating onset of 2-3 minutes is better explained by a relfex action than by an increase of either the vascular or interstitial space (3,6,7,8). To date, no data exist regarding the time course of the appearance of an ingested fluid in eccrine sweat.

The exercising adult can benefit from such data, as well. The loss of water by eccrine sweating is often greater than the intake of water by drinking (10). This is especially true when exercise intensities exceed 70% VO_2 max; at that point, gastric emptying appears to become an important limiting factor (4). One recent report, for example, described the unavoidable body weight deficits which a world class distance runner experienced during a hot, humid marathon competition (2), in spite of his efforts to prehydrate and to drink often. The common advice given to athletes and fitness enthusiasts is to drink during training and competition; yet, no data exist to indicate exactly how rapidly ingested water appears in eccrine

sweat or whether this advice is necessary during brief, intermittent exercise bouts. The advice to drink during exercise may offer protection against dehydration, but may offer little additional benefit from evaporative cooling. An improved understanding of the availability of ingested water for use by eccrine sweat glands may even modify current heat injury prevention doctrine.

Although eccrine sweat gland research is a relatively unexplored area of physiological research when compared to the recent progress being made in the study of other transport epithelia (12), the two aforementioned problems have not been investigated because the appropriate technology was not available and because radioactive tracers are undesireable in human studies. With the advent of stable isotope tracer methodology, stable isotopes such as ^2H or ^{18}O have proven to be suitable for measurements in a variety of biological fluids (14, Janghorbani unpublished observations.) Despite obvious value, stable isotope tracer methods have seldom been used in relation to the dynamics of body water during exercise, or in evaluating the function of eccrine sweat glands. Therefore, the purpose of the present investigation was to measure the rate of appearance of ingested water (using the stable isotope $H_2{}^{18}O$) in plasma and eccrine sweat, during intermittent exercise in the heat. Four males were exposed to an ambient temperature of $37.1^{\circ}C$ for 6 hours. Following the ingestion of an $H_2{}^{18}O$ labelled drink, blood and sweat samples were collected periodically. By measuring the isotope ratio of ^{18}O to ^{16}O, the rate of entry of the labelled drink was analyzed in eccrine sweat and in plasma.

Material & Methods

The subjects of this investigation were four healthy, unacclimatized males who received a thorough explanation of techniques, potential risks and benefits, prior to giving their voluntary written consent to participate. Physical charcteristics of subjects A, B, C, and D, respectively, were as follows: age - 37, 43, 47, 28 yr; height - 166, 178, 168, 184 cm; mass - 66.757, 58.822, 88.049, 94.250 Kg; surface area - 1.84, 1.67, 1.96, 2.14 m^2. Prior to arriving at the laboratory at 0800h, subjects had eaten no food for at least 2 hours. Electrocardiograph leads, a rectal thermister (8cm beyond the anal sphincter), and a forearm intravenous catheter were fitted after subjects entered the environmental chamber ($37.1 \pm 0.2^{\circ}C$ dry bulb, $31.3 \pm 0.2^{\circ}C$ wet bulb). Heart rate, rectal temperature, body weight ($\pm$ 10g), and blood pressure (sphygmomanometer) were recorded at regular intervals during each six hour exercise-heat exposure. Sweat rate was calculated by using body weight differences, corrected ($\pm$ 10g) for water and food intake as well as urine output. Exercise was utilized primarily as a stimulus for sweat production. All subjects exercised on a bicycle ergometer (Monark, model 868, Stockholm, Sweden) at work rates which were individually selected.

Baseline blood and sweat samples were obtained prior to the ingestion of 100g $H_2^{18}O$ (84.7% ^{16}O and 15.3% ^{18}O, Mound Laboratories, Miamisburg, OH). This bolus of labelled water was delivered via nasogastric tube and was rinsed with 100g distilled water. Water was consumed ad libitum and a light lunch and beverages were ingested between 3.5-4.5 hours postdosing. During the initial hour, blood and sweat samples were obtained at 10-15 min intervals, then at 60 min intervals for the remaining five hours. Venous blood was sampled from an antecubital vein in heparinized tubes, centrifuged, and the

plasma fraction was retained for isotopic analysis. Sweat (2-5ml) was scraped from the forehead and neck into clean polyethylene tubes. No attempt was made to collect uncontaminated samples because this sweat sampling technique was being evaluated for possible use in later field and laboratory studies.

The $^{18}O/^{16}O$ ratios in blood and sweat samples were analyzed using isotope ratio mass spectrometry (Isogas 903, Vacuum Generators, Manchester, U.K.). The rise in the $^{18}O/^{16}O$ ratio for either blood or sweat indicated the rate of entry of the ^{18}O enriched water for that fluid. Values were expressed as the g-atom ratio of $^{18}O/^{16}O$ by the use of a set of water standards spiked with varying but known amounts of $H_2^{18}O$. The measurement precision for $^{18}O/^{16}O$ ratios in deionozed water and various physiological fluids was evaluated at various levels of isotopic enrichment. The standard deviation for seven triplicate sets of these fluids was within the range 0.005 - 0.06%.

Results

FIGURE 1   Figure 1 depicts the duration of exercise and the hourly work output for each subject. Because bicycle ergometry was utilized to promote adequate sweat production, and because catheter patency, hourly sample collection and symptoms of heat illness were of primary importance, little effort was made to maintain a regular exercise schedule. For example, subjects A and B performed 43-46 min of exercise during the initial 60 min postdosing, while subjects C and D did no work on the ergometer. Subjects A and B also exercised during every hourly segment (exeption: hour 2 for subject B), whereas subjects C and D exercised only during hours 2, 3 and 4. Steady-state heart rates during exercise for subjects A, B, C and D ranged from: 102-120, 114-126, 88-100, 84-120 beats min^{-1}. Sweat rates were stable after the initial hour, and averaged 223 ± 25, 105 ± 35, 223 ± 51, and 260 ± 71 $ml \cdot m^{-2} \cdot h^{-1}$ (subjects A, B,

C and D respectively). The mean change in rectal temperature was $0.2 \pm 0.2^{\circ}C$ during these six hour trials.

TABLE 1   Table 1 describes baseline isotope ratios, prior to ingestion of the 100ml dose of ^{18}O labelled water. Sweat samples exhibited higher $^{18}O/^{16}O$ ratios than did corresponding plasma samples.

FIGURE 2   Figure 2 compares rate of entry of ^{18}O labelled water into plasma and sweat. The isotope ratio in plasma rose sharply, with peak appearance occurring at 21-28 min. A decline was observed in plasma until 60-150 min, depending on the subject. Thereafter, the plasma isotope ratio declined gradually for the remainder of the six hour observation period. Although the postdose isotopic enrichment was always higher in plasma, the rate of appearance of ^{18}O in sweat essentially paralelled its appearance in the plasma of all subjects. The time of ^{18}O peak appearance in sweat ranged from 18 min (subject D) to 45 min (subject C). Evidence of exogenous contamination (Fig. 2) appeared randomly at points in the sweat curves of subject C (275 min) and subject D (43 min).

Discussion

The isotopic enrichment of baseline sweat samples was greater than that of baseline plasma samples (Table 1). The exact reason for this observation in all four subjects is not evident, but these values are consistent with greater evaporation of the lighter isotope (^{16}O). An alternative explanation involves preferential secretion of ^{18}O by eccrine sweat glands. All plasma samples taken after the ingestion of labelled water (fig. 2) exhibited higher isotopic ratios than sweat. The rapid rise and subsequent fall of the plasma and sweat isotope ratios (fig. 2) represented first the rate of absorption and subsequently the equilibration of $H_2^{18}O$ in the vascular, interstitial and

intracellular compartments. Once the plasma and sweat curves had plateaued (approximately 60-120 min), a gradual decline was observed in the $^{18}O/^{16}O$ ratio (Fig. 2) for the remainder of the six hours. This gradual decline was probably due to four factors: (a) dilution with ad libitum liquid intake, (b) mixing of total body water with those compartments which are not readily accessible (e.g. synovial fluid, cerebrospinal fluid), (c) true expansion of body water from metabolically generated water, and (d) other anomalies such as isotope exhange between body water and the bicarbonate pool or possible exchange with nonaqueous oxygen.

The production of sweat in bursts shortly after water consumption has been observed by several investigators (3,6,7,8) but data are lacking to indicate whether these bursts represent a reflex response or an altered pressure/volume relationship within the circulatory system. The present investigation demonstrates that these theories may be examined using the stable isotope techniques described above. Oral administration of $H_2^{18}O$ would not be appropriate for short term measurements (e.g. 3 min postdose), however. Injection or infusion of the labelled water would be necessary.

Loss of water during exercise in the heat often exceeds the water replaced by drinking (2,10). The advice to consume water during exercise may offer protection against dehydration, but the time course of water availability for use by eccrine sweat glands was not known prior to this investigation. Figure 2 indicates that the ^{18}O labelled water appeared in sweat shortly after ingestion (9-18 min) and was maximally present in sweat at 21-45 minutes, depending on the subject. Because sweat rate may decline with severe dehydration (5,11,13), ingested water can clearly aid the exercising adult by maintaining skin wettedness during prolonged exercise. This is especially

significant considering the fact that evaporation of sweat may account for 85-96% of total heat dissipation during strenuous exercise in dry heat (1). Comparison of Figure 1 with Figure 2 indicates that the appearance of $H_2{}^{18}O$ in sweat was not influenced by the frequency, duration, or intensity of exercise, but apparently was solely a function of the rate of entry into the vascular space. The ^{18}O appearance curves of all subjects were strikingly similar, in spite of varying exercise regimes (Fig. 1) and ad libitum water intake.

Differences in isotopic ratios among various body fluids have been observed previously (14), and sweat is among the least preferred physiological matrices because of its potential for exogenous contamination (Janghorbani, unpublished observations). By sampling from enclosed skin surfaces, it should be possible to eliminate contamination. However, the data herein have successfully described the time course of fluid movements between the gastrointestinal tract, the vascular compartment and the interstitital space, and has clarified the physiological function of eccrine sweat glands. This investigation also has demonstrated that stable isotope methods can be valuable in measuring fluid dynamics during future heat acclimation, physical training, disease, and nutrition studies.

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Figure Titles

Figure 1 - Duration, hourly work output and total work performed by
subjects A - D.

Figure 2 - Appearance of $H_2{}^{18}O$ in plasma and sweat of subjects A - D.

- FIGURE 1 - 14

– FIGURE 2 – 15
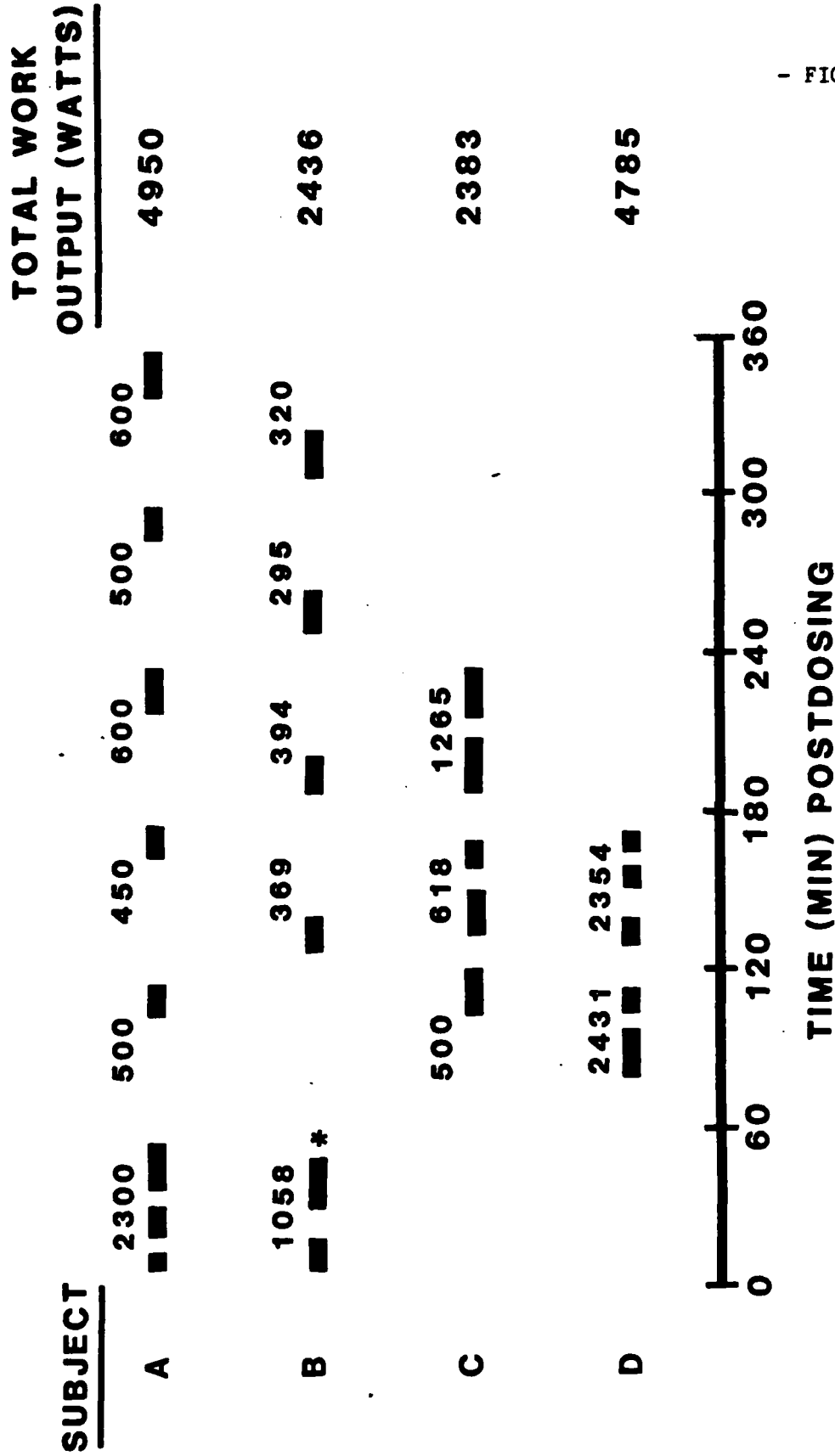
ISOTOPE RATIO ($^{18}O/^{16}O$ X 10^{-3})
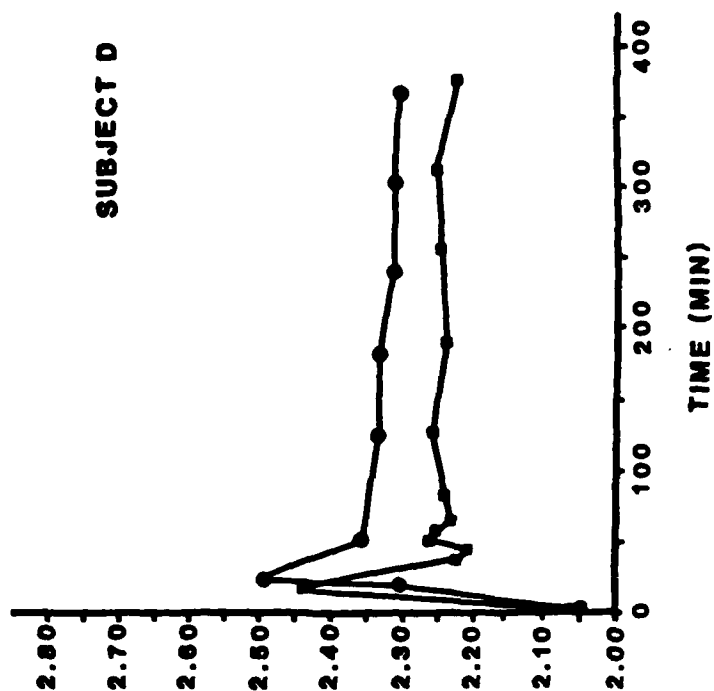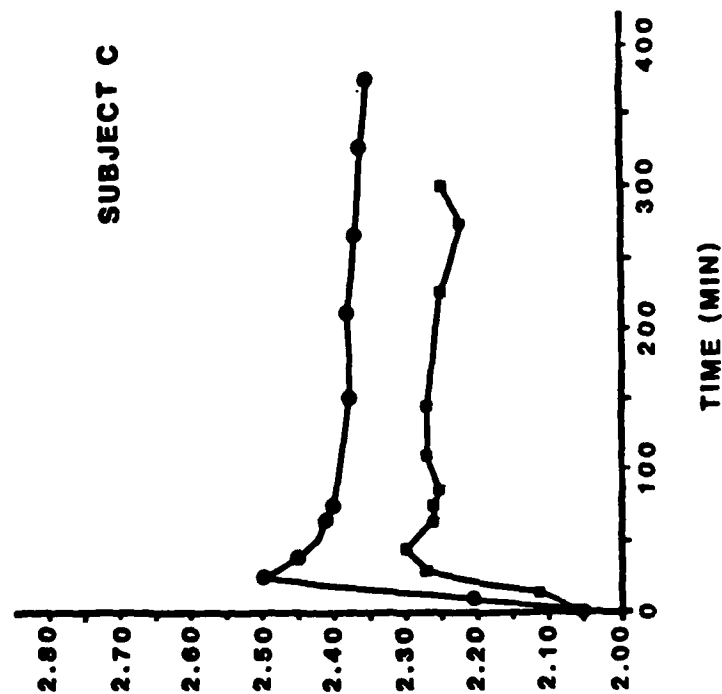
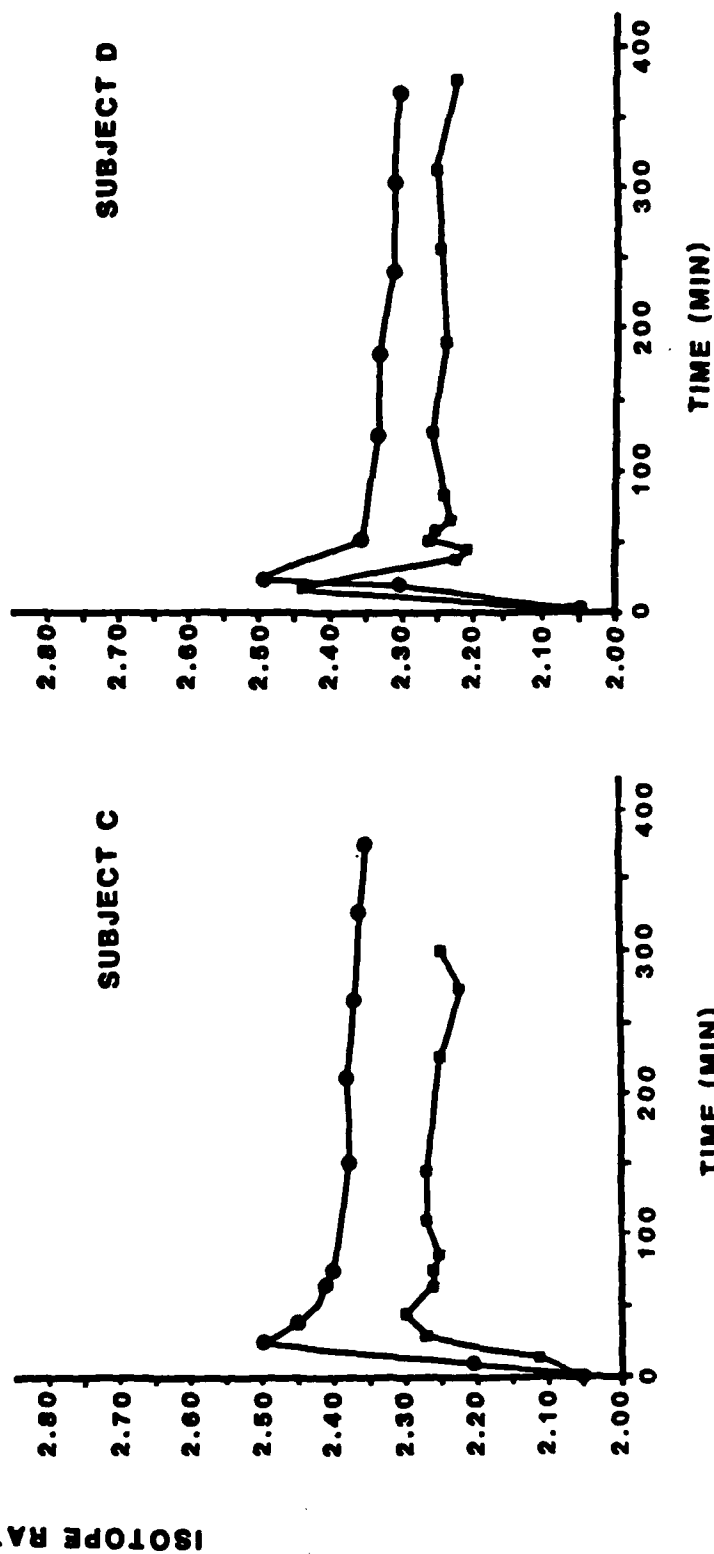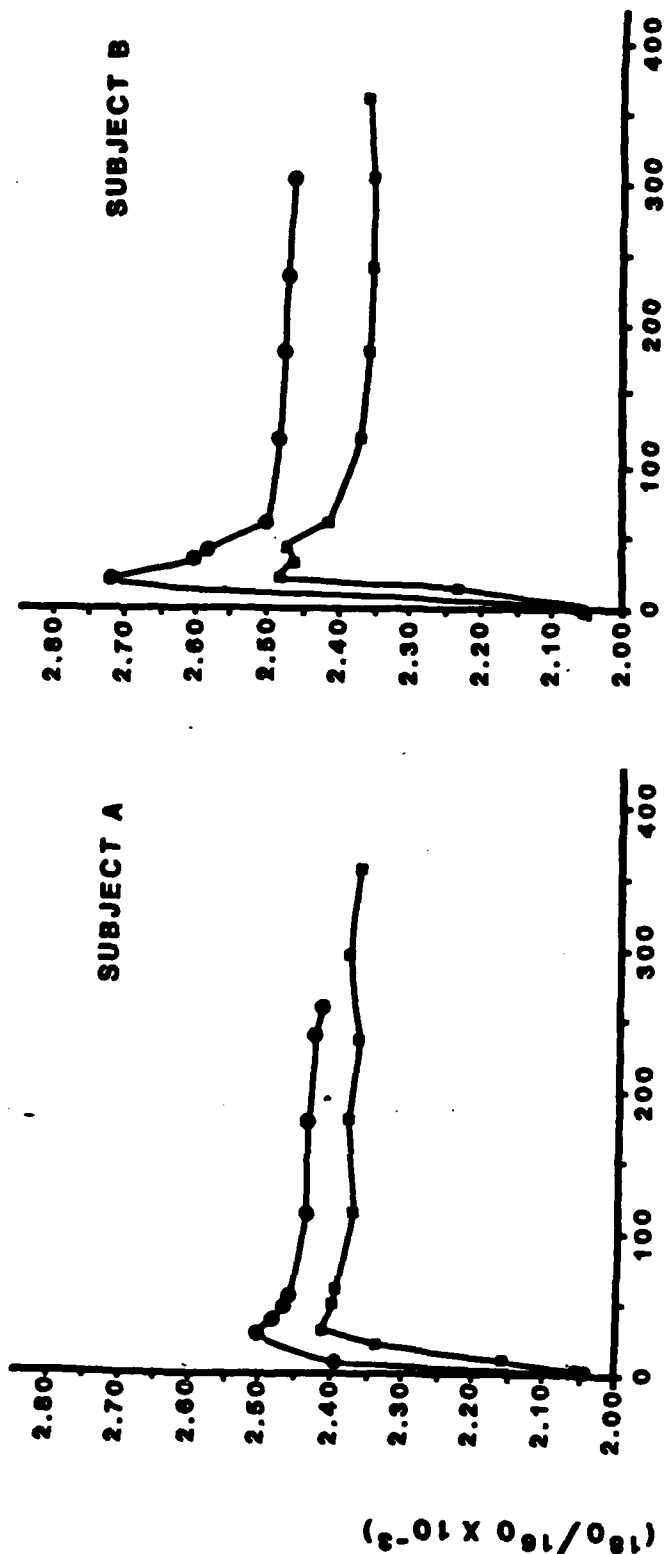Table 1 – Observed isotope ratios ($^{18}O/^{16}O$) in predose (0 min) samples of plasma and sweat.

Subject	Isotope ratio	
	Plasma	Sweat
A	2.0506×10^{-3}	2.0677×10^{-3}
B	2.0522×10^{-3}	2.0637×10^{-3}
C	2.0493×10^{-3}	2.0614×10^{-3}
D	2.0503×10^{-3}	2.0586×10^{-3}

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